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# Effects of Stratification and Temperature on Seed Germination Speed and Uniformity in Central Oregon Ponderosa Pine (*Pinus ponderosa* Dougl. ex Laws.)

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## Abstract

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Effects of stratification period and incubation temperature on seed germination speed and uniformity were investigated in a bulked seed lot of 200 ponderosa pine trees (*Pinus ponderosa* Dougl. ex Laws.) sampled from 149 locations in central Oregon. Mean rate of embryo development towards germination (1/days to 50 percent germination) and standard deviation of rate were estimated in a replicated, factorial experiment with four stratification periods (15, 30, 60, and 120 days) and four incubation temperatures (10, 15, 20, and 25 °C). Higher mean rate and standard deviation of rate, respectively, indicate fewer days to 50 percent germination (greater average speed) and less spread around the day of 50 percent germination (greater uniformity) if interpreted on the day scale (reciprocal of rate). Nearly all seeds germinated during an 80-day incubation period. Germination was complete with 60 days of stratification. Percentage of germination showed a peak at 20 °C incubation. Germination speed and uniformity increased with longer stratification and higher incubation temperature. Effects of stratification were greater at lower incubation temperatures, and effects of temperature were greater after shorter stratification. Longer stratification seemed to lower the minimum temperature requirement for germination. Multiple-regression equations accounted for more than 95 percent of the variation in means and standard deviations of rate. The discussion emphasizes practical implications for nursery managers who handle genetically diverse seed lots of central Oregon ponderosa pine.

Keywords: Seed, germination rate, stratification, temperature, ponderosa pine (central Oregon).



## Introduction

Seed germination speed and uniformity are important considerations in a tree improvement program. Rapid and uniform germination reduces the proportion of poorly developed seedlings, thereby minimizing the loss of genetic variation during the operational nursery phase of a tree improvement program (Campbell and Sorensen 1984). Nursery managers can increase germination speed and uniformity in ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.), and many other forest tree species, by subjecting seed lots to a longer period of moist chilling (stratification) before sowing (Schopmeyer 1974). The optimal stratification period, however, depends on seedbed temperature (Vegis 1964); and the response to stratification-temperature differs among seed sources in ponderosa pine (Curtis 1955, Woods and Blake 1981, Weber 1988<sup>1</sup>) and other tree species (Schopmeyer 1974). The nursery manager, therefore, must choose a stratification period that is both appropriate for local seedbed temperatures and satisfies the stratification-temperature requirements of genetically diverse seed lots.

In this paper, we investigate the general effects of stratification period and incubation temperature on germination speed and uniformity in central Oregon ponderosa pine. The discussion emphasizes practical implications for nursery managers working with genetically diverse seed lots of central Oregon ponderosa pine.

## Methods

### Sample Collection

Mature cones were collected in 1981 from 200 trees sampled from 149 locations in central Oregon. A sampling intensity of one location per one-half of a township (~ 2300 hectares) was planned in the Malheur, Ochoco, and Deschutes National Forests (NF), but this intensity was not possible because of low cone production in some areas. Most sample locations were in the Ochoco NF (mainly in Big Summit, Paulina, Maury, and Snow Mountain Ranger Districts) and in the Malheur NF (mainly in Burns, Long Creek, and Bear Valley Ranger Districts). One tree was sampled at 98 locations, and two trees, at least 100 meters apart, were sampled at 51 locations. This sampling scheme was designed primarily for an investigation of geographic variation in several seed and seedling characters.

About 70 percent of the sampled trees were select trees. Seeds from select trees had been collected for reforestation because the trees exhibited phenotypic characteristics in the field that were desirable for timber production. The other trees were randomly selected from those that produced an adequate cone supply and were relatively easy to sample. Select trees were generally less than 75 years old when sampled. Age of the other trees was not determined, but they were similar in size to the select trees.

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<sup>1</sup> Weber, J.C.; Sorensen, F.C. [In preparation] Geographic variation in seed germination speed in central Oregon ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.). Being prepared at: U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station, Forestry Sciences Laboratory, 3200 S.W. Jefferson Way, Corvallis, Oregon 97331.

Equal numbers of full seeds from the 200 trees were mixed together in a bulked seed lot for this study. Seeds were extracted after cones had dried to a constant weight. Empty seeds and those with a poorly developed embryo and gametophyte were discarded (identified by weight and x ray). Lots of 50 seeds each were then subsampled from the bulked seed lot. Lots were stored at  $-10^{\circ}\text{C}$  until stratified for the germination tests (autumn 1982).

## **Experimental Procedures**

The experimental design was a factorial with four stratification periods (15, 30, 60, and 120 days), four incubation temperatures after stratification (10, 15, 20, and  $25^{\circ}\text{C}$ ), and four replications. For each stratification period, 16 lots of 50 seeds each (four incubation temperatures by four replications) were removed from cold storage and transferred to separate mesh bags. Seedlots were soaked in aerated, distilled water ( $\sim 22^{\circ}\text{C}$ ) for 24 hours, dipped in fungicide for 30 seconds ( $\sim 4$ -grams Captan powder per liter water), and then air-dried for 30 minutes. The 16 seed lots were then placed into a polyethylene bag containing moist paper towels and stratified at  $2-3^{\circ}\text{C}$  for the designated time. Bags were rotated daily to minimize potential microenvironmental effects in the cold room.

All stratification treatments terminated simultaneously. The 64 seed lots were then transferred to petri dishes containing moist vermiculite covered by moist filter paper. To ensure aeration within the petri dishes, plastic ties were used to maintain a gap between the dish and lid. Petri dishes were placed in four germinators, each calibrated to the designated temperature (24-hour light period). Sixteen dishes (four stratification periods by four replications) were randomized on one shelf of each germinator. Dishes were systematically rotated daily to minimize potential microenvironmental effects in the germinators. Distilled water was added as needed to maintain moist filter paper.

Germinated seeds were counted on a variable schedule, beginning after 1 day and ending after 80 days in the germinator. Seeds were considered to have germinated when their radicle appeared  $\sim 1.0$  millimeter long. Counts were more frequent at the beginning of the incubation period when germination was peaking; nearly half of the 50 total counts were made in the first week. Some seeds germinated during the 120-day stratification period, and they were included in the first count. Seeds destroyed by fungi were recorded and removed. Ungerminated seeds were cut open at the end of incubation; full seeds, considered potentially viable, and fungi-infected seeds were counted.

## **Analyses of Germination Data**

The mean and standard deviation of embryo development rate towards germination were estimated for each seed lot by using a procedure suggested by Campbell and Sorensen (1979). Rate is the reciprocal of days to germination ( $1/\text{days}$ ); if a seed requires, for example, 4 days to germinate, its embryo development rate is 0.25 units per day. Mean rate is the reciprocal of days to 50 percent germination, and standard deviation of rate is the standard deviation around mean rate. We analyzed germination data on the rate scale rather than the day scale because the distribution was closer to normal on the rate scale.



The mean and standard deviation of rate were estimated from germinated seeds only; ungerminated seeds within a seed lot were not included in the estimation. Rates were generally lower if ungerminated seeds were included in the estimation, but the same basic relations with stratification period and incubation temperature were observed.

The estimation procedure is described in detail elsewhere (Campbell and Sorensen 1979) and is briefly summarized below. The 50 counting times were combined into 18-30 time intervals (depending on the seed lot) during which at least one seed germinated. Cumulative percentage of germination over the time intervals was computed for each seed lot (among germinated seeds only). Cumulative percentages were transformed to probits, and time intervals were converted to rate intervals (1/days). Probits were regressed on rates. The mean and standard deviation of rate were estimated from the regression coefficient and intercept by a maximum-likelihood method.<sup>2</sup>

Average germination speed and uniformity were defined as days to 50 percent germination rather than as rates. Although germination was analyzed on a rate scale, we chose the reciprocal, day scale for interpretation. We defined average germination speed as days to 50 percent germination (reciprocal of mean rate), and uniformity as the spread in days to 50 percent germination (reciprocal of standard deviation of rate). A higher mean rate reflects fewer days to 50 percent germination (greater average speed), and a higher standard deviation of rate reflects less spread around the day of 50 percent germination (greater uniformity). Because these are reciprocal variables, greater spread on the rate scale (higher standard deviation of rate) translates into less spread on the day scale.

Mean and standard deviation of rate were transformed to logarithms to satisfy the assumptions of analysis of variance. On the untransformed rate scale, distributions of means and standard deviations exhibit heterogeneous variances and positive skewness. After transformation ( $\text{Log}_{10}[\text{rate by } 10^4]$ ), means are negatively skewed ( $P < 0.05$ ), but otherwise both means and standard deviations satisfy the assumptions of analysis of variance.

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<sup>2</sup> Grpnorm computer program and documentation. On file with: R.K. Campbell and F.C. Sorensen, U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station, Forestry Sciences Laboratory, 3200 S.W. Jefferson Way, Corvallis, Oregon 97331.

Effects of stratification, temperature, and their interaction on the mean and standard deviation of rate were tested by analysis of variance. Stratification period (days) and incubation temperature ( $^{\circ}\text{C}$ ) were also transformed to  $\text{Log}_{10}$  to linearize the relations (Sorensen 1983). All effects were random in a partially nested model, which included the following sources of variation: stratification period, incubation temperature, stratification by temperature interaction, replication nested within temperature, and residual (interaction between stratification period and replication nested within temperature). Stratification and temperature mean squares (MS) were tested over the interaction MS, and the MS for interaction and replication within temperature were tested over the residual MS. Main effects were partitioned into linear, quadratic, and cubic contrasts, and the interaction was partitioned into nine contrasts involving linear, quadratic, and cubic terms (all single degree of freedom).

Multiple-regression analysis was used to investigate response surfaces of the mean and standard deviation of rate in relation to stratification and temperature. All variables were transformed as in analysis of variance: stratification and temperature,  $\text{Log}_{10}$  (days or  $^{\circ}\text{C}$ ); mean and standard deviation,  $\text{Log}_{10}$  (rate by  $10^4$ ). All sources of variation that were significant in analysis of variance were included as independent variables in an initial model. The model was then reduced by backward elimination; independent variables were removed one by one, depending on their incremental contribution to the regression sum-of-squares. The reduced model that explained most variation and included only significant independent variables ( $P < 0.05$ ) was then tested for lack of fit using the sum-of-squares of replications within the 16 stratification-temperature treatments as an estimate of pure error (Draper and Smith 1966). If lack of fit was significant, additional terms were added and the procedure repeated.

## Results

Nearly all full seeds germinated during the 80-day incubation period (table 1). All full seeds germinated if stratified at least 60 days. Some seeds even germinated during the 120-day stratification treatment. Percentage of germination over all stratification periods was highest at  $20^{\circ}\text{C}$  incubation. Percentage of germination was surprisingly high at  $10^{\circ}\text{C}$  and low at  $25^{\circ}\text{C}$  incubation in seed lots stratified 15 days.

**Table 1—Summary of percent germination observed among potentially viable seeds after 4 stratification periods (15, 30, 60, and 120 days) and 4 incubation temperatures ( $10^{\circ}\text{C}$ ,  $15^{\circ}\text{C}$ ,  $20^{\circ}\text{C}$ , and  $25^{\circ}\text{C}$ )<sup>a</sup>**

| Stratification<br>period | 10 °C incubation    |          |       | 15 °C incubation |          |       | 20 °C incubation |          |       | 25 °C incubation |          |       |       |
|--------------------------|---------------------|----------|-------|------------------|----------|-------|------------------|----------|-------|------------------|----------|-------|-------|
|                          | Total               | Days to: |       | Total            | Days to: |       | Total            | Days to: |       | Total            | Days to: |       | Total |
|                          |                     | 90       | Total |                  | 90       | Total |                  | 90       | Total |                  | 90       | Total |       |
| Days                     | ----- Percent ----- |          |       |                  |          |       |                  |          |       |                  |          |       |       |
| 15                       | 92.9                | 76       | 80    | 79.0             | >80      | 80    | 95.7             | 37       | 73    | 84.2             | >80      | 80    | 87.9  |
| 30                       | 95.3                | 66       | 80    | 96.1             | 18       | 67    | 100.0            | 7        | 48    | 97.3             | 14       | 80    | 97.1  |
| 60                       | 100.0               | 21       | 80    | 100.0            | 6        | 54    | 100.0            | 4        | 27    | 100.0            | 4        | 27    | 100.0 |
| 120                      | 100.0               | 11       | 24    | 100.0            | 5        | 22    | 100.0            | 3        | 15    | 100.0            | 4        | 43    | 100.0 |
| Total percent            | 97.1                |          |       | 93.8             |          |       | 98.9             |          |       | 95.3             |          |       | 96.6  |

<sup>a</sup> Sample sizes for percentages are 178 to 196 for each stratification-temperature combination, 733 to 765 for each stratification or temperature treatment, and 3000 over all treatments. Days to 90 percent and total percent germination are estimated by linear interpolation in some cases. Seeds consumed by fungi (~ 4 percent) are not included in calculations.

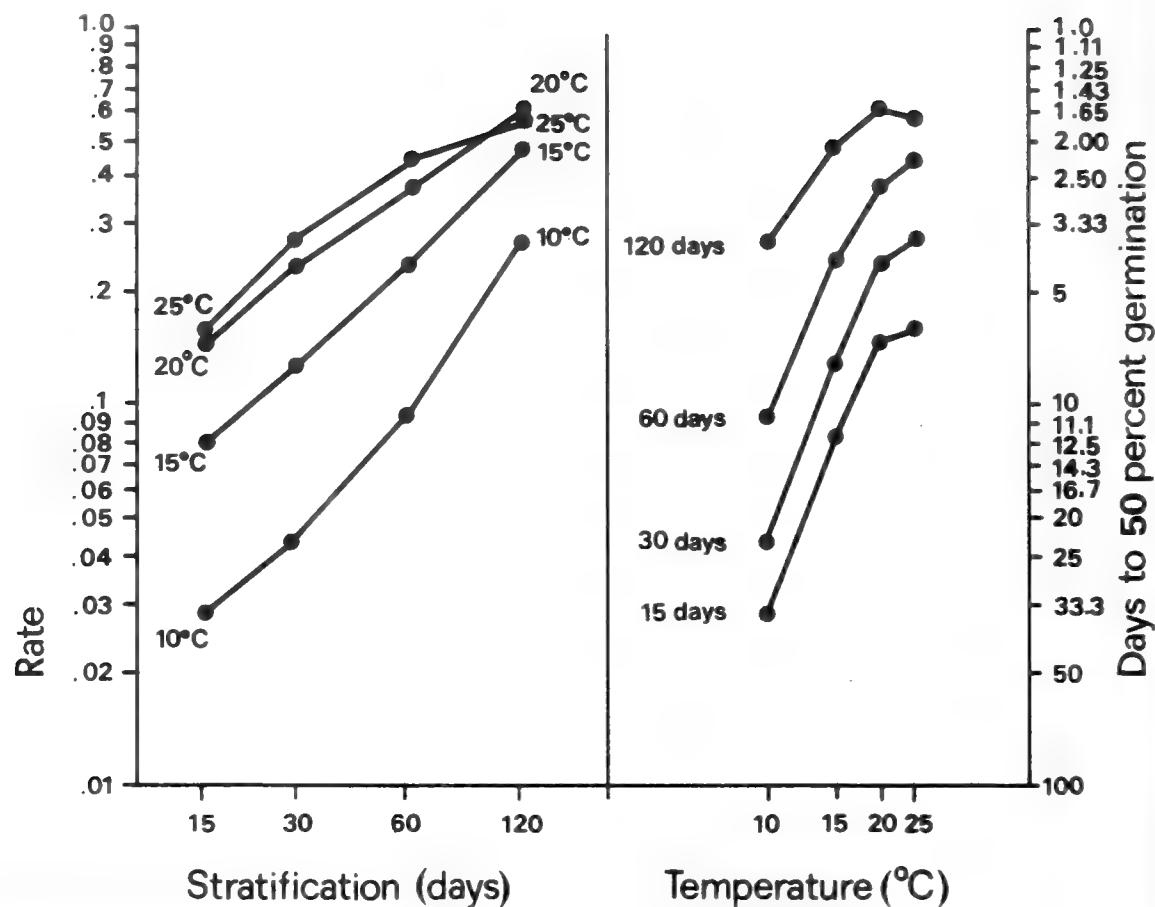
The distribution of germination times is positively skewed. Fifty percent of the seeds may germinate in a very short time, depending on stratification and temperature. The remaining 50 percent, however, germinate over a longer time; and the time between, for instance, 90 percent and 100 percent is longer than that between 80 percent and 90 percent. For example, 50 percent germination was observed in the 60 day-10 °C treatment by ~ 11 days of incubation (fig. 1 mean, days to 50 percent germination on right scale); but 90 percent germination required ~ 21 days, and 100 percent germination required ~ 80 days (table 1). Average germination speed and uniformity differ among ponderosa pine trees in central Oregon (Weber 1988), so late-germinating seeds in this study represent only a fraction of the 200 sample trees.

The observed mean and standard deviation of rate increase, in general, with longer stratification periods and higher incubation temperatures (fig. 1). That is, longer stratification periods and higher incubation temperatures generally produce greater average germination speed (fewer days to 50 percent germination) and more uniform germination within seed lots (less spread around the day of 50 percent germination). The mean and standard deviation are positively correlated (Pearson  $r = 0.91$ ,  $N = 64$ , and  $P < 0.001$ ). Note that a unit change in rate at the lower end of the rate scale causes a relatively larger difference in days to 50 percent germination than does a unit change at the higher end of the rate scale.

Effects of stratification and temperature are not simply linear and additive (fig. 1), but linear contrasts of stratification and temperature account for most of the variation in the standard deviation of rate and especially in the mean of rate (table 2). The proportion of variation due to linear contrasts is larger for stratification than temperature, especially among standard deviations. The proportion of variation due to interaction, mainly linear by linear interaction, is considerably larger among standard deviations than among means. The stratification by temperature interaction is quite apparent in plots of standard deviations, especially between the 60- and 120-day stratification treatments (fig. 1).

The selected multiple-regression equations account for more than 95 percent of the variation in the mean and standard deviation of rate (table 3). Stratification has a relatively larger effect than temperature on both mean and standard deviation, judging by the magnitude of standardized partial regression coefficients, but interaction terms are highly significant. Some terms that are not significant in analysis of variance are included in the regression equations to improve the fit. Because terms are tested by different F-ratios in analysis of variance and multiple-regression analysis, the significance of F-ratios differs.

## MEAN



## STANDARD DEVIATION

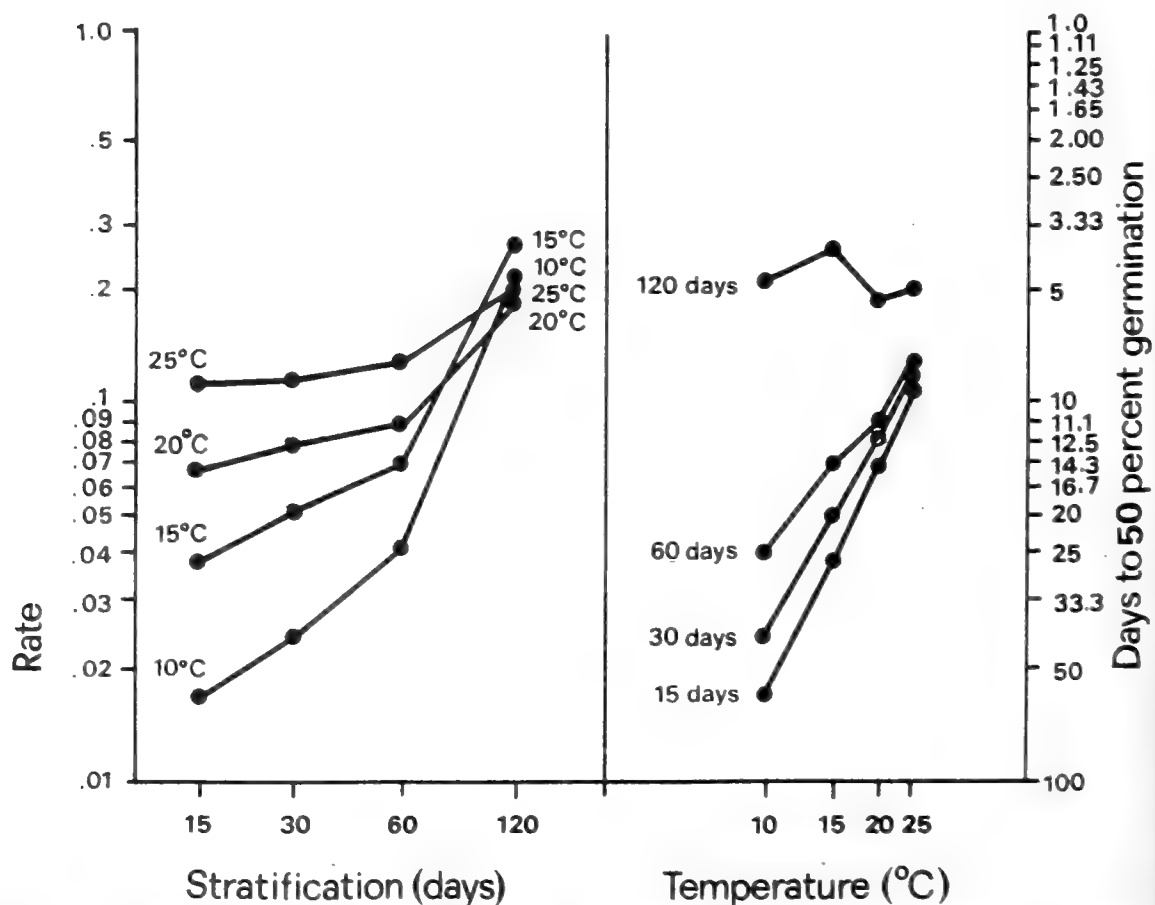


Figure 1—Germination mean and standard deviation observed after four stratification periods (15, 30, 60, and 120 days) and four incubation temperatures (10, 15, 20, and 25 °C). Treatment averages are connected by lines (means above, standard deviations below) and are plotted against stratification period for each incubation temperature (left), and against temperature for each stratification period (right). The rate scale is shown on the left; and the reciprocal of rate, days to 50 percent germination, is shown on the right. All variables are plotted on a Log<sub>10</sub> scale. Means and standard deviations are estimated from germinated seeds only.

**Table 2—Analysis of variance of germination mean rate and standard deviation of rate among 4 stratification periods (15, 30, 60, and 120 days) and 4 incubation temperatures (10, 15, 20, and 25 °C)<sup>a</sup>**

| Source of variation                   | Degrees of freedom | Mean rate     |           |                         | Standard deviation of rate |           |                         |
|---------------------------------------|--------------------|---------------|-----------|-------------------------|----------------------------|-----------|-------------------------|
|                                       |                    | Mean square   | F-ratio   | Proportion of variation | Mean square                | F-ratio   | Proportion of variation |
| <b>Stratification:</b>                |                    |               |           |                         |                            |           |                         |
| Linear (SL)                           | 1                  | 4.80577       | 129.33*** | 0.509                   | 3.54578                    | 31.54***  | 0.471                   |
| Quadratic (SQ)                        | 1                  | .01500        | .40       | .002                    | .56392                     | 5.02      | .075                    |
| Cubic (SC)                            | 1                  | .00045        | .01       | .000                    | .09051                     | .81       | .012                    |
| <b>Temperature:</b>                   |                    |               |           |                         |                            |           |                         |
| Linear (TL)                           | 1                  | 3.94445       | 106.15*** | .417                    | 2.01066                    | 17.89**   | .267                    |
| Quadratic (TQ)                        | 1                  | .18766        | 5.05      | .020                    | .00160                     | .01       | .000                    |
| Cubic (TC)                            | 1                  | .00791        | .21       | .001                    | .02385                     | .21       | .003                    |
| <b>Stratification by temperature:</b> |                    |               |           |                         |                            |           |                         |
| SLxTL                                 | 1                  | .24359        | 83.42***  | .026                    | .88396                     | 167.73*** | .117                    |
| SLxTQ                                 | 1                  | .00189        | .65       | .000                    | .00487                     | .92       | .001                    |
| SLxTC                                 | 1                  | .00228        | .78       | .000                    | .00888                     | 1.69      | .001                    |
| SQxTL                                 | 1                  | .08377        | 28.69***  | .009                    | .09995                     | 18.97***  | .013                    |
| SQxTQ                                 | 1                  | .00051        | .17       | .000                    | .00138                     | .26       | .000                    |
| SQxTC                                 | 1                  | .00037        | .13       | .000                    | .00002                     | .00       | .000                    |
| SCxTL                                 | 1                  | .00033        | .11       | .000                    | .00750                     | 1.42      | .001                    |
| SCxTQ                                 | 1                  | .00001        | .00       | .000                    | .00519                     | .98       | .001                    |
| SCxTC                                 | 1                  | .00165        | .57       | .000                    | .00002                     | .00       | .000                    |
| <b>Replication within temperature</b> | 12                 | .00396        | 1.35      | .005                    | .00784                     | 1.49      | .012                    |
| <b>Residual</b>                       | 36                 | .00292        |           | .011                    | .00527                     |           | .025                    |
| <b>Total</b>                          | <b>63</b>          | <b>.14997</b> |           | <b>1.000</b>            | <b>.11955</b>              |           | <b>1.000</b>            |

<sup>a</sup> Asterisks indicate significance of F-ratios: \*\*\*P≤0.001, \*\*P≤0.01, \*P≤0.05, no asterisk P>0.05. Proportion of variation is the proportion of total variation due to each variance component. All variables are transformed: stratification Log<sub>10</sub> (days); temperature Log<sub>10</sub> (°C); mean and standard deviation, Log<sub>10</sub> (rate by 10<sup>4</sup>). Means and standard deviations are estimated from germinated seeds only.

**Table 3—Multiple-regression equations relating variation in germination mean rate and standard deviation of rate to stratification period (15 to 120 days) and incubation temperature (10 to 25 °C)<sup>a</sup>**

| Term                           | Mean rate                      |   |          | Standard deviation of rate     |   |          |
|--------------------------------|--------------------------------|---|----------|--------------------------------|---|----------|
|                                | Partial regression coefficient | Standardized partial regression coefficient | F-ratio  | Partial regression coefficient | Standardized partial regression coefficient | F-ratio  |
| Stratification:                |                                |   |          |                                |   |          |
| Linear (SL)                    | -8.8796509                     | -7.777                                      | 19.33**  |                                |   |          |
| Quadratic (SQ)                 | 3.4394374                      | 9.839                                       | 30.94*** | -3.0713982                     | -9.841                                      | 16.30*** |
| Cubic (SC)                     |                                |   |          | 1.6658761                      | 13.462                                      | 30.59*** |
| Temperature:                   |                                |   |          |                                |   |          |
| Linear (TL)                    | 4.3160524                      | 1.672                                       | 7.30**   | -3.3649959                     | -1.460                                      | 4.97*    |
| Quadratic (TQ)                 | -3.1160984                     | -2.887                                      | 63.61*** |                                |   |          |
| Stratification by temperature: |                                |   |          |                                |   |          |
| SLxTL                          | 7.5027406                      | 9.352                                       | 20.81*** | 8.3965539                      | 11.722                                      | 19.31*** |
| SQxTL                          | -2.6834701                     | -9.853                                      | 28.39*** | -3.2980641                     | -13.563                                     | 31.69*** |
| Intercept                      | 1.8260673                      |   | 1.18     | 1.8304677                      |   | 7.64**   |

<sup>a</sup> Asterisks indicate significance of F-ratios: \*\*\*P≤0.001, \*\*P≤0.01, \*P≤0.05, no asterisk P>0.05. Degrees of freedom for testing individual terms = 1, 57 for mean and 1, 58 for standard deviation. Equation F-ratio (degrees of freedom), proportion of variation explained by equation, and standard deviation from regression, respectively = 524.25\*\*\* (6, 57), 0.983, and 0.054 for mean rate; and 243.93\*\*\* (5, 58), 0.955, and 0.077 for standard deviation of rate. All variables are transformed: stratification Log<sub>10</sub> (days); temperature Log<sub>10</sub> (°C); mean and standard deviation, Log<sub>10</sub> (rate by 10<sup>4</sup>). Means and standard deviations are estimated from germinated seeds only.

The general effects of stratification and temperature on germination speed and uniformity, predicted from equations in table 3, are briefly summarized below. Because the equations account for so much of the variation, the reader can refer to plots of observed treatment averages in fig. 1 to see the general response surfaces. The effect of stratification on average germination speed (mean, days to 50 percent germination) is greater at lower incubation temperatures. For example, increasing stratification from 30 to 60 days reduces days to 50 percent germination from ~ 24 to 11 at 10 °C, and from ~ 4 to 3 at 20 °C. Similarly, the effect of incubation temperature is greater after shorter stratification periods. For example, increasing temperature from 10 to 20 °C reduces days to 50 percent germination from ~ 24 to 4 if stratified 30 days, and from ~ 11 to 3 if stratified 60 days. With 120 days of stratification, days to 50 percent germination differ by only ~ 3 days between 10 and 25 °C incubation. Effects of stratification and temperature on germination uniformity (standard deviation, days to 50 percent germination) are similar to the effects on average speed.

The regression equations summarize a general relation illustrated in fig. 1: longer stratification lowers the minimum temperature requirement for germination, thereby resulting in more rapid and uniform germination at lower temperatures (Vegis 1964). For example, average germination speed is highest at 25 °C with 60 days stratification, but highest at ~ 20 °C with 120 days stratification (mean, fewer days to 50 percent germination). Average uniformity of germination is also highest at 25 °C with 60 days stratification, but highest at ~ 15 °C with 120 days stratification (standard deviation, fewer days to 50 percent germination).



For a given stratification period and incubation temperature, we can use the equations in table 3 to estimate the time interval for a specified percentage of germination in central Oregon ponderosa pine. The estimation procedure is described in an appendix with two examples that emphasize the effect of stratification period on germination speed and uniformity.

## Discussion

Inadequate stratification of genetically diverse seed lots can delay emergence in the nursery bed and cause it to be less uniform and complete compared to adequately stratified seed lots. In addition to interfering with good nursery culture, delayed emergence may result in selection in the nursery against genotypes with greater stratification requirements (Campbell and Sorensen 1984). This selection is undesirable because it reduces the parentage (gene pool) represented in seedling populations and may reduce the range of potentially adaptive variation in some plantation environments. For example, the timing of seed germination may be genetically correlated with bud-burst time and/or other phenological traits of the seedling. Forest microclimates are extremely variable in space and time (Lee 1978). To maintain plantation adaptability, seedling populations used for reforestation should include a broad range of variation in phenological and other potentially adaptive traits (Silen 1982).

There is indirect evidence that chilling requirements for ponderosa pine seed germination vary geographically in central Oregon and that the variation is related to the relative severity of summer drought (Weber 1988, see footnote 1). Provenances from more mesic locations seem to have greater average chilling requirements than do provenances from locations with shorter, drought-limited growing seasons; and the range in chilling requirements seems to be associated with the range in precipitation among provenance locations. For example, the range in chilling requirements seems to be greater on the eastern face of the Cascade Range (in the Deschutes National Forest), where precipitation decreases sharply from west to east, than in regions further east of the Cascade Range (Ochoco and Malheur National Forests), where the climate is generally drier and more continental.

These variation patterns in chilling requirements, if correct, have two practical implications: (1) the 30-day stratification period typically used for central Oregon ponderosa pine seed lots may be adequate for the most xeric breeding zones but will be inadequate for some seed lots from many of the more mesic zones and for zones with a larger range in precipitation; and (2) unless optimal stratification periods are determined for each breeding zone, stratification should be increased for all zones, especially the more mesic zones and those with larger ranges in precipitation.

Longer stratification periods are necessary for genetically diverse seed lots sown into colder nursery beds to achieve rapid and uniform germination. Increasing the stratification period lowers the minimum temperature requirement for seed germination, resulting in more rapid and uniform germination at lower incubation temperatures. This is an important point because Oregon nurseries typically sow seed lots into open nursery beds in April, when soil temperatures are well below optimum for germination. For example, if seed lots are stratified for 30 days, 90 percent germination would require only ~ 7 days of incubation at 20 °C (table 1), but this is an unrealistically high soil temperature for early spring in Oregon. At more realistic temperatures of 15 °C and below, 90 percent germination would require ~ 18 days of incubation at 15 °C and approximately 66 days at 10 °C. With 60 days of stratification, 90 percent

germination would require only ~ 6 days of incubation at 15 °C and only ~ 21 days at 10 °C. With 120 days of stratification, 90 percent germination would require even less time at these low incubation temperatures.

This paper describes general relations between stratification-temperature and seed germination in central Oregon ponderosa pine. As discussed above, chilling requirements seem to show certain broad geographic variation patterns; so breeding zones will differ in the percentage, speed and uniformity of germination, depending on their genetic composition and the particular stratification-temperature conditions.

In summary, we make the following recommendations for stratifying seed lots of central Oregon ponderosa pine: (1) stratification should be increased to at least 60 days; (2) increasingly longer stratification is necessary for nurseries located in areas with colder spring temperatures; (3) increasingly longer stratification is necessary for breeding zones that are more mesic or variable in precipitation. These recommendations are very general, and they are based on germination studies under controlled laboratory conditions. The relations between seed germination and stratification-temperature are being investigated under more realistic nursery-bed conditions. Studies of geographic variation in chilling requirements are necessary to refine these recommendations.

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## Appendix

We describe a procedure for estimating the time interval for a specified percentage of germination in central Oregon ponderosa pine by using the equations in table 3. Before describing the procedure, we will emphasize several points. The procedure estimates time intervals for percentage of germination, not seedling emergence. Estimation is limited to the tested ranges in stratification period (15 to 120 days) and incubation temperature (10 to 25 °C). Estimates are for central Oregon as a whole, not for seed lots from individual trees. Equations were developed from a study of germination under controlled conditions, so estimates may differ under nursery bed conditions. Estimates are not free of error. Equations predict mean and standard deviation of rate with a certain probability of error. In addition, we assume that rates are normally distributed to estimate time intervals; accuracy of estimates depends on how closely the actual distribution approximates this distribution.

The general procedure includes three steps: (1) estimate the mean and standard deviation of rate by using the equations in table 3, (2) estimate the range in rates for a specified percentage of germination by using properties of the normal distribution, and (3) change the range from rate to days. We illustrate the procedure by estimating the time interval for 80 percent and 95 percent germination in seeds given two different stratification periods (28 and 84 days) and then incubated at 12 °C.

**Step 1: estimating mean and standard deviation of rate.** Predicted values are affected by rounding-off error: use the complete partial regression coefficients in table 3, and use the same number of digits for Log<sub>10</sub> of days and °C. Do not round off while using the equation.

Estimating mean rate: Log<sub>10</sub> (rate by 10,000) =  
 – [8.8796509 by Log<sub>10</sub> (days)],  
 + [3.4394374 by Log<sub>10</sub> (days) by Log<sub>10</sub> (days)],  
 + [4.3160524 by Log<sub>10</sub> (°C)],  
 – [3.1160984 by Log<sub>10</sub> (°C) by Log<sub>10</sub> (°C)],

$$\begin{aligned}
&+ [7.5027406 \text{ by } \log_{10}(\text{days}) \text{ by } \log_{10}(^{\circ}\text{C})], \\
&- [2.6834701 \text{ by } \log_{10}(\text{days}) \text{ by } \log_{10}(\text{days}) \text{ by } \log_{10}(^{\circ}\text{C})], \text{ and} \\
&+ 1.8260673.
\end{aligned}$$

Estimating standard deviation of rate:  $\log_{10}(\text{rate by } 10,000) =$

$$\begin{aligned}
&- [3.0713982 \text{ by } \log_{10}(\text{days}) \text{ by } \log_{10}(\text{days})], \\
&+ [1.6658761 \text{ by } \log_{10}(\text{days}) \text{ by } \log_{10}(\text{days}) \text{ by } \log_{10}(\text{days})], \\
&- [3.3649959 \text{ by } \log_{10}(^{\circ}\text{C})], \\
&+ [8.3965539 \text{ by } \log_{10}(\text{days}) \text{ by } \log_{10}(^{\circ}\text{C})], \\
&- [3.2980641 \text{ by } \log_{10}(\text{days}) \text{ by } \log_{10}(\text{days}) \text{ by } \log_{10}(^{\circ}\text{C})], \text{ and} \\
&+ 1.8304677.
\end{aligned}$$

The equations give  $\log_{10}(\text{rate by } 10,000)$ , so we convert to rate in two steps:  
 (1) antilog of equation result = (rate by 10,000), and  
 (2) (rate by 10,000)/10,000 = rate.

Calculations for the 28-day per 12  $^{\circ}\text{C}$  example:  
 $\log_{10}(28) = 1.4471580$  and  $\log_{10}(12) = 1.0791812$ .

Mean:  $\log_{10}(\text{rate by } 10,000) =$

$$\begin{aligned}
&- [8.8796509 \text{ by } 1.4471580] + [3.4394374 \text{ by } 1.4471580 \text{ by } 1.4471580], \\
&+ [4.3160524 \text{ by } 1.0791812] - [3.1160984 \text{ by } 1.0791812 \text{ by } 1.0791812], \\
&+ [7.5027406 \text{ by } 1.4471580 \text{ by } 1.0791812], \\
&- [2.6834701 \text{ by } 1.4471580 \text{ by } 1.4471580 \text{ by } 1.0791812], \text{ and} \\
&+ 1.8260673 = 2.8600834.
\end{aligned}$$

Convert to rate:  
 (1) antilog of 2.8600834 =  $10^{2.8600834} = 724.6$  (rounded-off) = rate by 10,000, and  
 (2)  $724.6/10,000 = 0.07246$  = mean rate.

Standard deviation:  $\log_{10}(\text{rate by } 10,000) =$

$$\begin{aligned}
&- [3.0713982 \text{ by } 1.4471580 \text{ by } 1.4471580], \\
&+ [1.6658761 \text{ by } 1.4471580 \text{ by } 1.4471580 \text{ by } 1.4471580], \\
&- [3.3649959 \text{ by } 1.0791812], \\
&+ [8.3965539 \text{ by } 1.4471580 \text{ by } 1.0791812], \\
&- [3.2980641 \text{ by } 1.4471580 \text{ by } 1.4471580 \text{ by } 1.0791812], \text{ and} \\
&+ 1.8304677 = 2.4748806.
\end{aligned}$$

Convert to rate:  
 (1) antilog of 2.4748806 =  $10^{2.4748806} = 298.5$  = rate by 10,000, and  
 (2)  $298.5/10,000 = 0.02985$  = standard deviation of rate.

Calculations for the 84-day per 12  $^{\circ}\text{C}$  example:  
 The only difference in the calculations is that  $\log_{10}(\text{days}) = 1.9242793$ .  
 Mean:  $\log_{10}(\text{rate by } 10,000) = 3.3608210$ ; rate = 0.22952.  
 Standard deviation:  $\log_{10}(\text{rate by } 10,000) = 2.9534483$ ; rate = 0.08984.

**Step 2: estimating the range in germination rates for a specified percentage of germination by using properties of the normal distribution.** If germination rate has a normal (bell-shaped) distribution, a certain percentage of rates occurs within a certain number of standard deviation units above and below the mean. Standard deviation units for areas of the normal curve are available in many statistical tables (for example, Rohlf and Sokal 1969, p. 157-158). For our examples, ~ 80 percent of rates would occur within mean  $\pm 1.282$  standard deviations, and ~ 95 percent would occur within mean  $\pm 1.96$  standard deviations if rates are normally distributed.

Calculations for the 28-day per 12 °C example:

~ 80 percent of rates would occur within  $0.07246 \pm (1.282 \text{ by } 0.02985)$ .  
Range =  $0.07246 \pm 0.03827 = 0.03419$  to  $0.11073$ .

~ 95 percent of rates would occur within  $0.07246 \pm (1.960 \text{ by } 0.02985)$ .  
Range =  $0.07246 \pm 0.05851 = 0.01395$  to  $0.13097$ .

Calculations for the 84-day per 12 °C example:

~ 80 percent of rates would occur within  $0.22952 \pm (1.282 \text{ by } 0.08984)$ .  
Range =  $0.11435$  to  $0.34469$ .

~ 95 percent of rates would occur within  $0.22952 \pm (1.96 \text{ by } 0.08984)$ .  
Range =  $0.05343$  to  $0.40561$ .

**Step 3. changing the range from rate to days.** Compute the reciprocal of rate (that is,  $1/\text{rate}$ ) to convert rate to days. Larger rates mean fewer days.

Calculations for the 28-days per 12 °C example:

Mean rate =  $0.07246$ ; reciprocal of rate = 13.8 days to 50 percent germination.

~ 80 percent of rates range from  $0.03419$  to  $0.11073$ ; reciprocal values = 29.25 and 9.03, respectively, so the estimated time interval for 80 percent germination extends from 9.03 to 29.25 days. The distribution on the day scale is not symmetric around the mean (very positive skew).

~ 95 percent of rates range from  $0.01395$  to  $0.13097$ ; reciprocal values = 71.68 and 7.64, respectively, so the estimated time interval for 95 percent germination extends from 7.64 to 71.68 days.

Calculations for the 84-day per 12 °C example:

Mean rate =  $0.22952$ ; reciprocal of rate = 4.36 days to 50 percent germination.

~ 80 percent of rates range from  $0.11435$  to  $0.34469$ , so the estimated time interval extends from 2.90 to 8.75 days.

~ 95 percent of rates range from  $0.05343$  to  $0.40561$ , so the estimated time interval extends from 2.47 to 18.72 days.



**Weber, John C.; Sorensen, Frank C. 1990.** Effects of stratification and temperature on seed germination speed and uniformity in central Oregon ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.). Res. Pap. PNW-RP-429. Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station. 13 p.

Effects of stratification period and incubation temperature on seed germination speed and uniformity were investigated in a bulked seed lot of 200 ponderosa pine trees (*Pinus ponderosa* Dougl. ex Laws.) sampled from 149 locations in central Oregon. Mean rate of embryo development towards germination (1/days to 50 percent germination) and standard deviation of rate were estimated in a replicated, factorial experiment with four stratification periods (15, 30, 60, and 120 days) and four incubation temperatures (10, 15, 20, and 25 °C). Higher mean rate and standard deviation of rate, respectively, indicate fewer days to 50 percent germination (greater average speed) and less spread around the day of 50 percent germination (greater uniformity) if interpreted on the day scale (reciprocal of rate). Nearly all seeds germinated during an 80-day incubation period. Germination was complete with 60 days of stratification. Percentage of germination showed a peak at 20 °C incubation. Germination speed and uniformity increased with longer stratification and higher incubation temperature. Effects of stratification were greater at lower incubation temperatures, and effects of temperature were greater after shorter stratification. Longer stratification seemed to lower the minimum temperature requirement for germination. Multiple-regression equations accounted for more than 95 percent of the variation in means and standard deviations of rate. The discussion emphasizes practical implications for nursery managers who handle genetically diverse seed lots of central Oregon ponderosa pine.

**Keywords:** Seed, germination rate, stratification, temperature, ponderosa pine (central Oregon).

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